DNA, Genes and their Regulation

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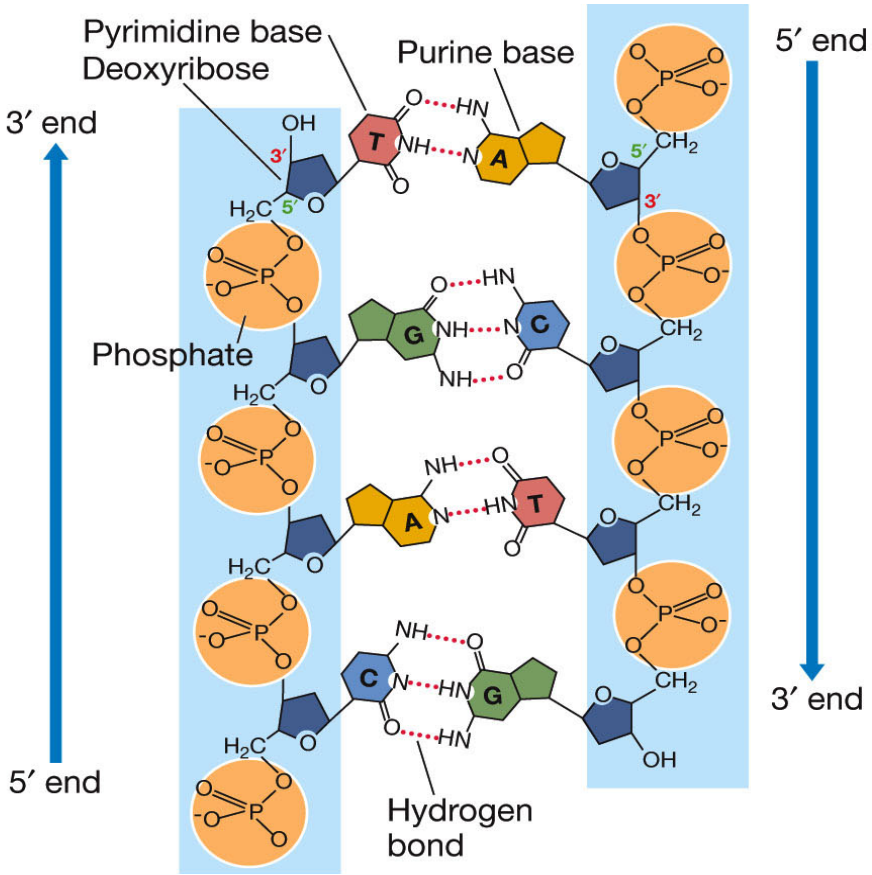
Learning Objectives

After this lecture, you should be able to

- Account for the structure of DNA and RNA including their similarities and differences, and how complementary base pairing can take place between two DNA strands or between a DNA and an RNA strand. Describe how DNA is packed into chromosomes.
- Describe the Central Dogma of Molecular Biology, in particular transcription. Deduce the sequence of a pre-mRNA molecule that has been transcribed from a given gene.
- Sketch the structure of a eukaryotic gene and explain the difference between exons and introns.
- Describe how gene expression is regulated in eukaryotes emphasizing the many different levels this can be achieved on.

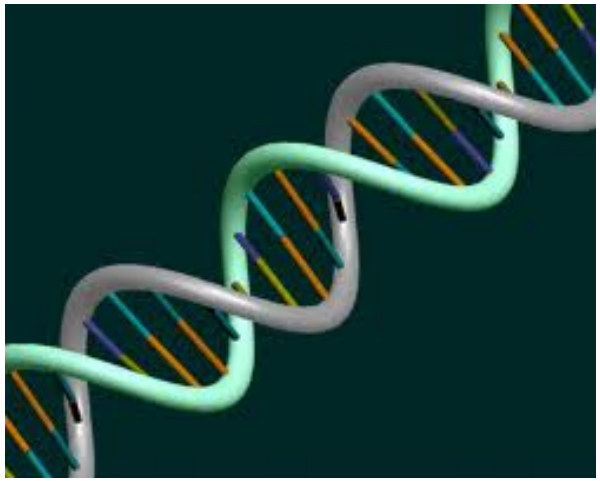
DNA

Deoxyribose Nucleic Acid



LIFE 8e, Figure 3.24

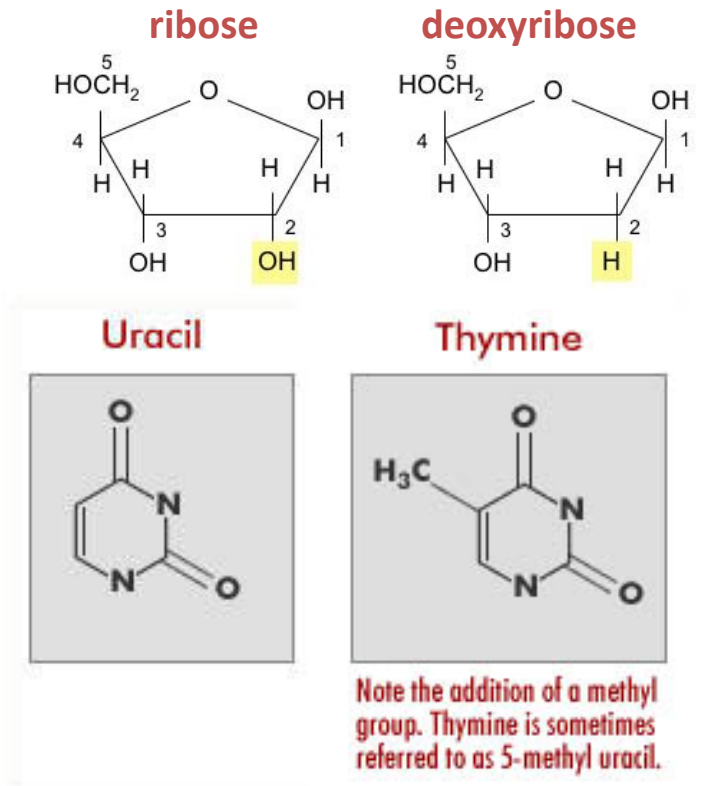
- Deoxyribose, phosphate, base (**A**denine, **T**hymine, **G**uanine, **C**ytosine)
- Double helix: Via hydrogen bonds, A pairs with T and C with G.
- The two strands are antiparallele.

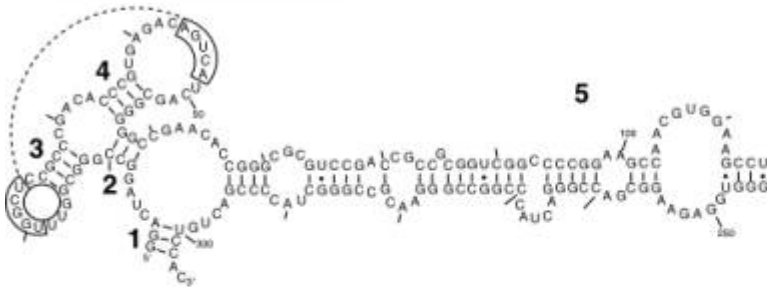


RNA

RiboNucleic Acid

- **RNA** differs from DNA in three ways:
  - RNA is usually single-stranded
  - The sugar is *ribose*, not deoxyribose
  - RNA contains the base *uracil* (U) instead of thymin (T)
- **RNA** can basepair with single-stranded DNA (adenin pairs with uracil instead of with thymin)
- An **RNA**-strand can fold and basepair with itself (creating a secondary structure)



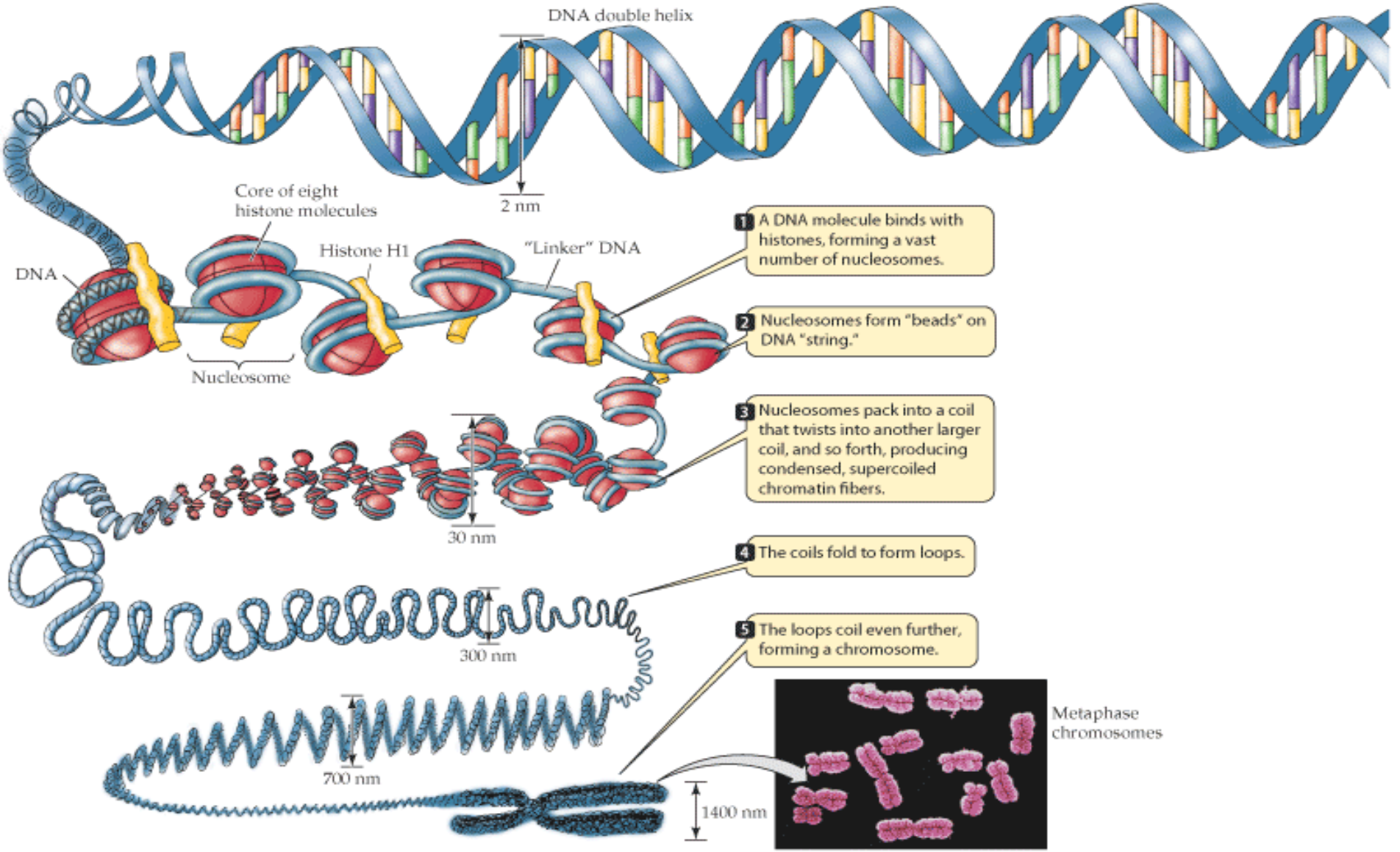


Genes are sequences of DNA

>gi|6007800:215-1075 Escherichia coli beta-lactamase variant TEM-1D (blaTEM-1D) gene, complete cds

5’ATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTC
ACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAGCT
GGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTT
AAAGTTCTGCTATGTGGTGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATAC
ACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGT
AAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATC
GGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACCCGCCTTGATCGTTGGG
AACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAAC
GTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAG
GCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTG
GAGCCGGTGAGCGTGGATCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGT
AGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCC
TCACTGATTAAGCATTGGTAA 3’

DNA is packed into chromosomes



Learning objective

Be able to account for the structure of DNA and RNA including their similarities and differences and how complementary base pairing can take place between two DNA strands or between a DNA and an RNA strand. Describe how DNA is packed into chromosomes.

Typical exam question

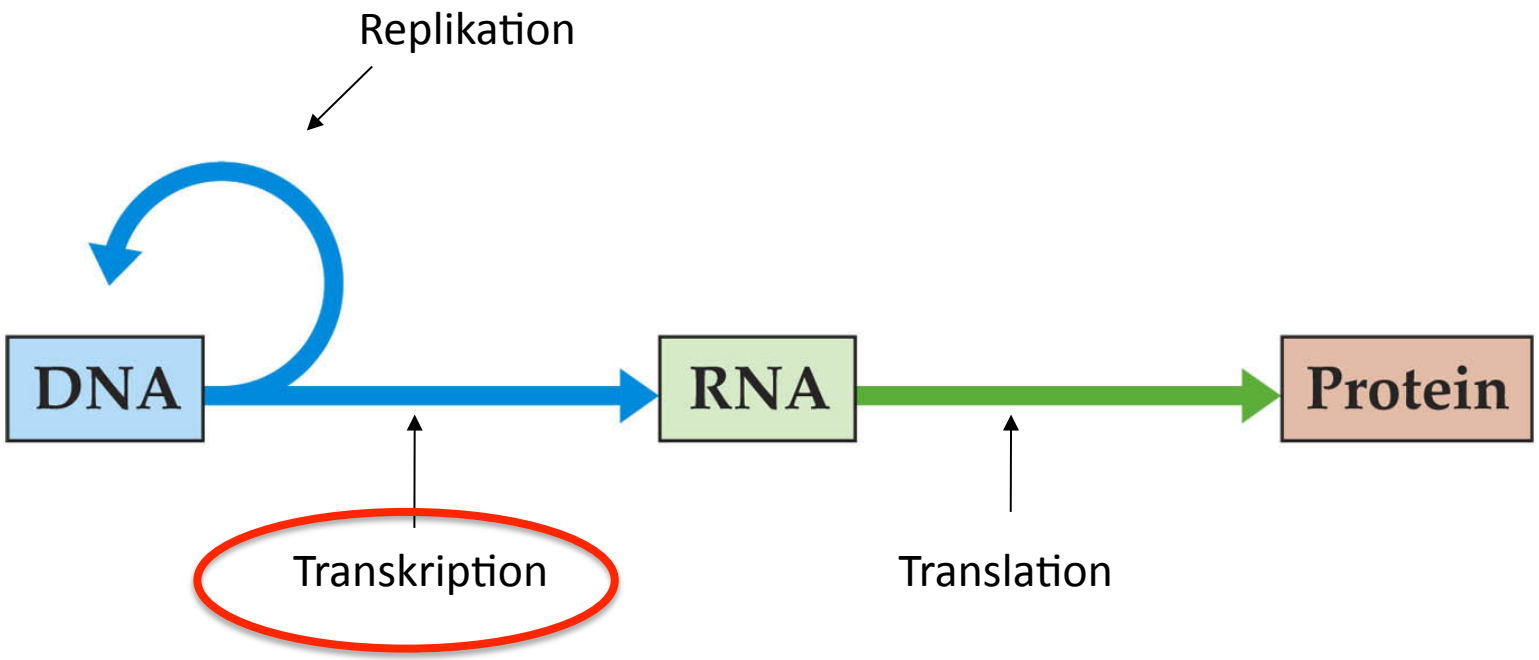
Q: Below is shown a single stranded DNA molecule. Which of the strands shown in a. - e. is the complementary DNA strand?

Single stranded DNA molecule: 5’ ATGCCCGGG 3’

a. 3’ ATGCCCGGG 5’
b. 5’ CCCGGGCAT 3’
c. 3’ CCCGGGCAT 5’
d. 3’ UACGGGCCC 5’
e. 5’ TACGGGCCC 3’

The Central Dogma of Molecular Biology

- Genetic information is transferred from DNA to RNA to protein. (Almost) never in the opposite direction (Francis Crick).



Transcription

Template or Coding Strand?

DNA

>gi|6007800:215-1075 Escherichia coli beta-lactamase variant TEM-1D (blaTEM-1D) gene, complete cds

ATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTC
ACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAGCT
GGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTT
AAAGTTCTGCTATGTGGTGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATAC
ACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGT
AAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATC
GGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACCCGCCTTGATCGTTGGG
AACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAAC
GTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAG
GCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTG
GAGCCGGTGAGCGTGGATCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGT
AGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCC
TCACTGATTAAGCATTGGTAA

transcription

>blaTEM-1D mRNA

RNA

AUGAGUAUUCAACAUUUCCGUGUCGCCCUUAUUCCCUUUUUUGCGGCAUUUUGCCUUCCUGUUUUUGCUC
ACCCAGAAACGCUGGUGAAAGUAAAAGAUGCUGAAGAUCAGUUGGGUGCACGAGUGGGUUACAUCGAGCU
GGAUCUCAACAGCGGUAAGAUCCUUGAGAGUUUUCGCCCCGAAGAACGUUUUCCAAUGAUGAGCACUUUU
AAAGUUCUGCUAUGUGGUGCGGUAUUAUCCCGUGUUGACGCCGGGCAAGAGCAACUCGGUCGCCGCAUAC
ACUAUUCUCAGAAUGACUUGGUUGAGUACUCACCAGUCACAGAAAAGCAUCUUACGGAUGGCAUGACAGU
AAGAGAAUUAUGCAGUGCUGCCAUAACCAUGAGUGAUAACACUGCGGCCAACUUACUUCUGACAACGAUC
GGAGGACCGAAGGAGCUAACCGCUUUUUUGCACAACAUGGGGGAUCAUGUAACCCGCCUUGAUCGUUGGG
AACCGGAGCUGAAUGAAGCCAUACCAAACGACGAGCGUGACACCACGAUGCCUGCAGCAAUGGCAACAAC
GUUGCGCAAACUAUUAACUGGCGAACUACUUACUCUAGCUUCCCGGCAACAAUUAAUAGACUGGAUGGAG
GCGGAUAAAGUUGCAGGACCACUUCUGCGCUCGGCCCUUCCGGCUGGCUGGUUUAUUGCUGAUAAAUCUG
GAGCCGGUGAGCGUGGAUCUCGCGGUAUCAUUGCAGCACUGGGGCCAGAUGGUAAGCCCUCCCGUAUCGU
AGUUAUCUACACGACGGGGAGUCAGGCAACUAUGGAUGAACGAAAUAGACAGAUCGCUGAGAUAGGUGCC
UCACUGAUUAAGCAUUGGUAA

Learning objective

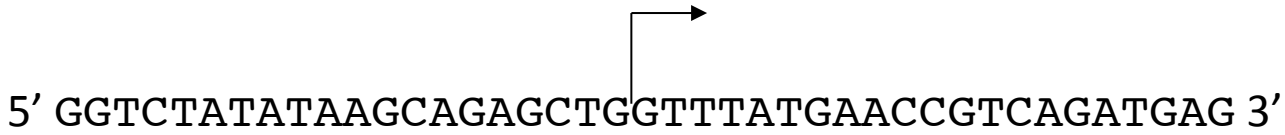
Be able to describe the Central Dogma of Molecular Biology, in particular transcription. Deduce the sequence of a pre-mRNA molecule that have been transcribed from a given gene

Typical exam questions

Q: The "central dogma" of molecular biology states that

a. information flow between DNA, RNA, and protein is reversible.
b. information flow in the cell is unidirectional, from protein to DNA.
c. information flow in the cell is unidirectional, from DNA to protein.
d. the DNA sequence of a gene can be predicted if we know the amino acid sequence of the protein it encodes.

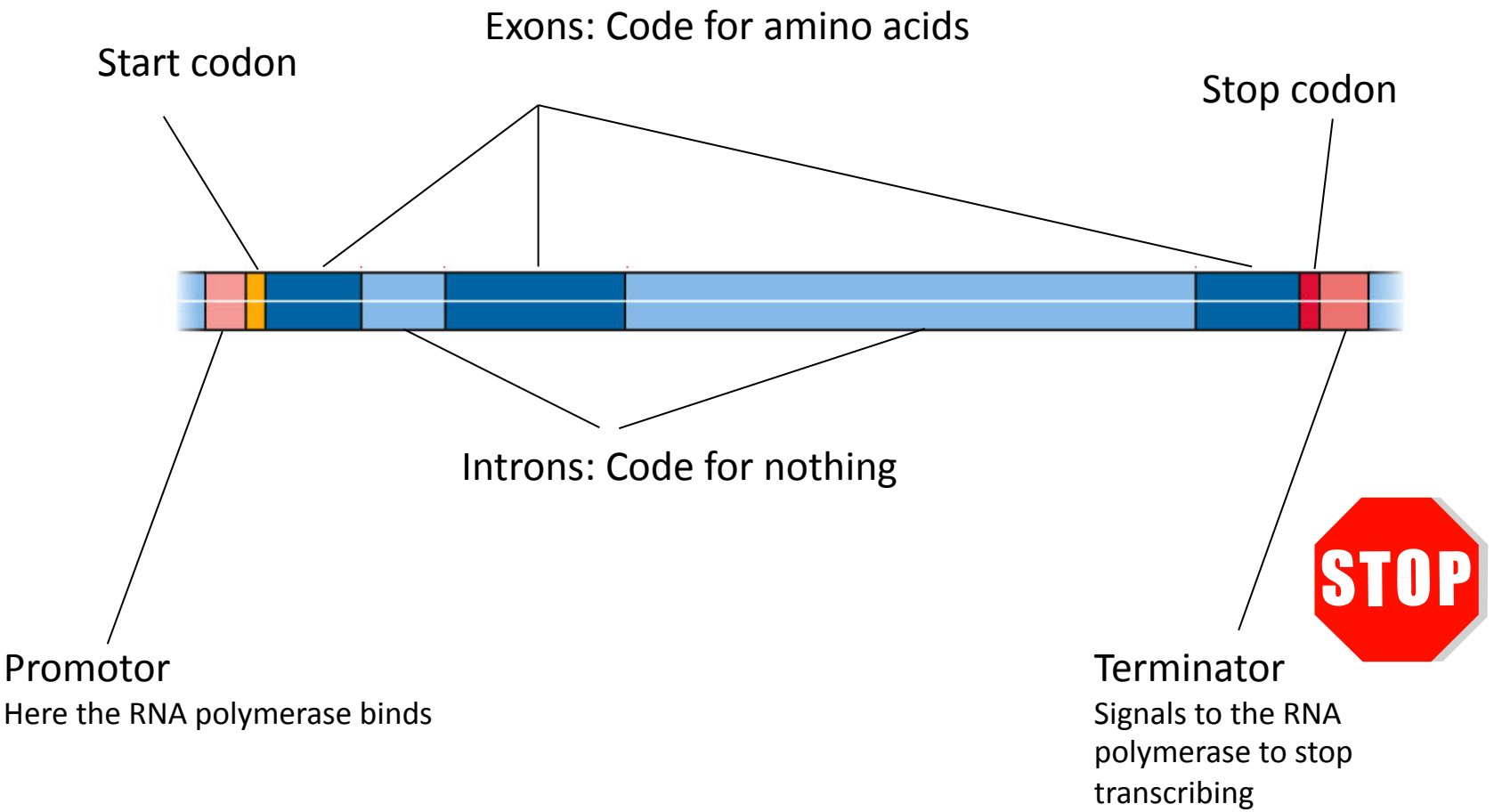
Q: Below, the **coding** strand of a DNA molecule is shown. The arrow indicates where, and in which direction transcription occurs. Write the resulting mRNA molecule



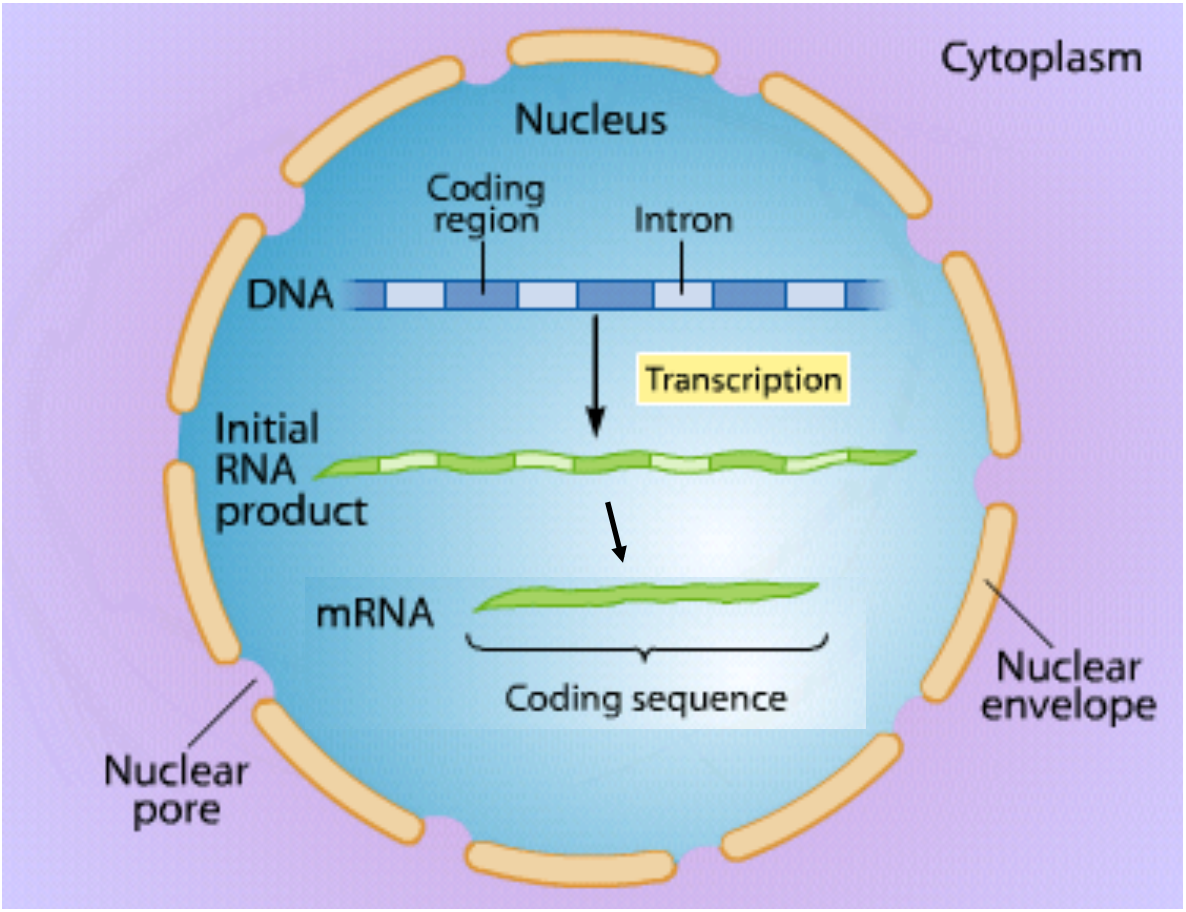
a. 5' GUUUAUGAACCGUCAGAUGAG 3'
b. 3' GUUUAUGAACCGUCAGAUGAG 5'
c. 3' CAAATACTTGGCAGTCTACTC 5'

BREAK

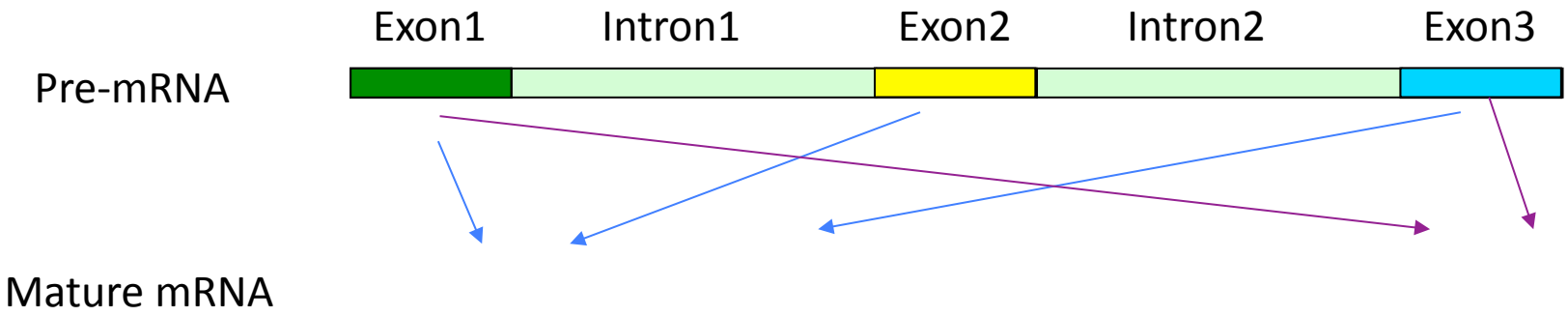
Structure of eukaryotic protein-coding genes



Structure of eukaryotic protein-coding genes



Alternative splicing



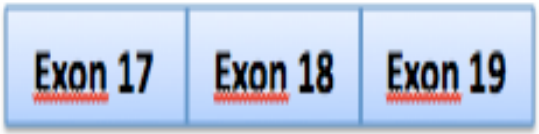
Learning objective

Be able to sketch the structure of a eukaryotic gene and explain the difference between exons and introns

Typical exam question

The *RB* gene contains 27 exons and 26 introns. Below, exon no. 17-19 and intron no. 17-19 are shown schematically. Sketch the mature mRNA molecule with regards to these exons/introns (you may assume that there is no alternative splicing).

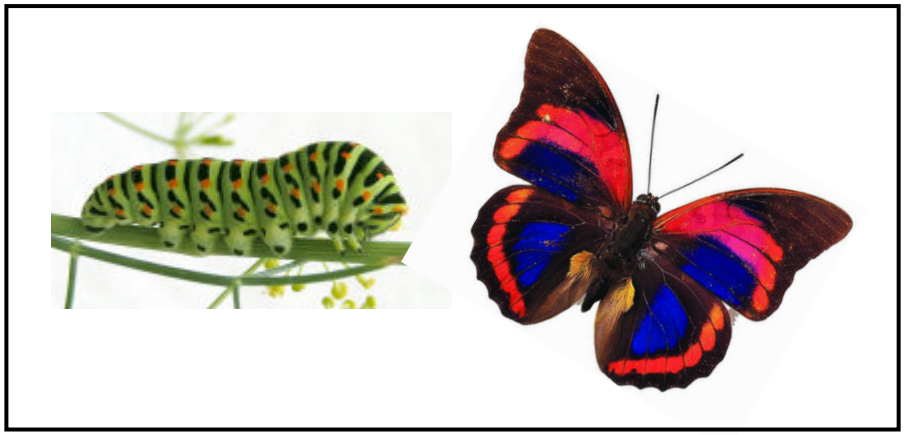
Answer:



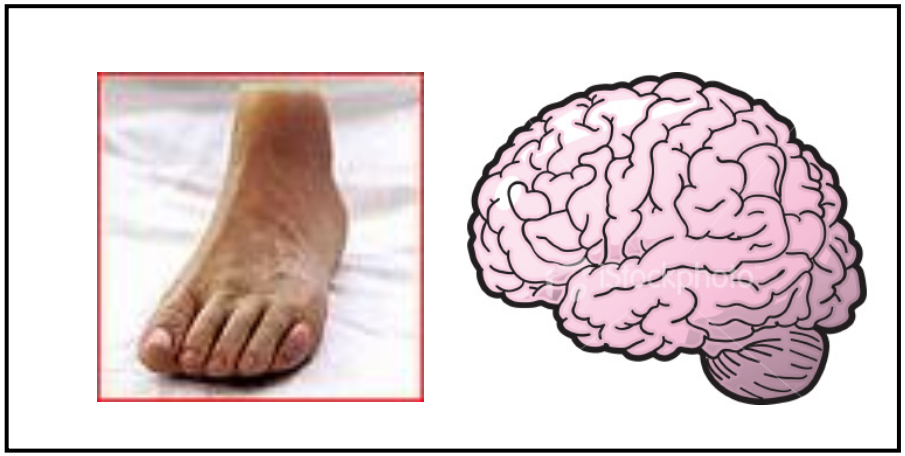
(Only exons are present in the mature mRNA. Introns are spliced out)

Regulating gene expression in multicellular organisms

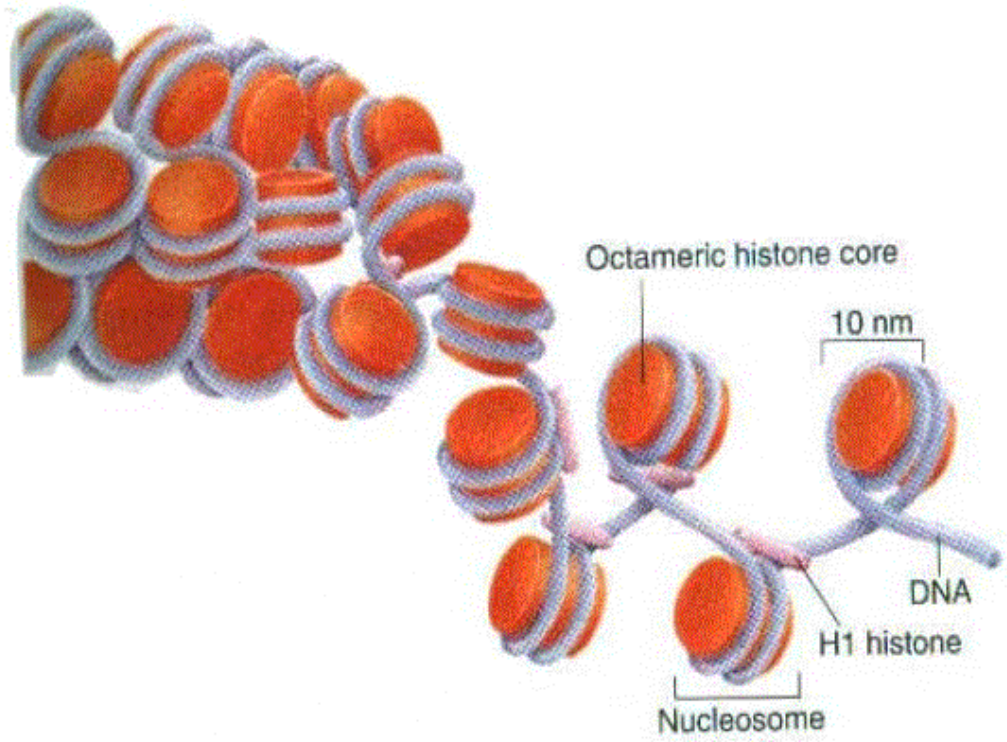
Same genome – different expression at different stages



Same genome – different expression in different places



Chromatin (DNA+histones)

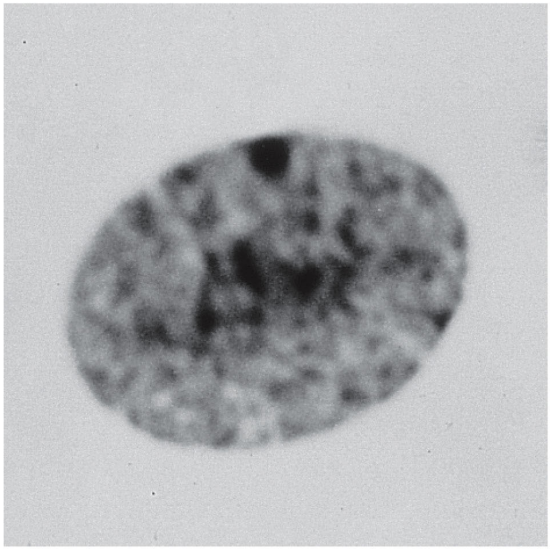


vsrp.uhnres.utoronto.ca/research1.htm

Euchromatin: Lightly packed chromatin. The genes are transcribed.
Heterochromatin: Tightly packed chromatin. The genes are normally not transcribed.

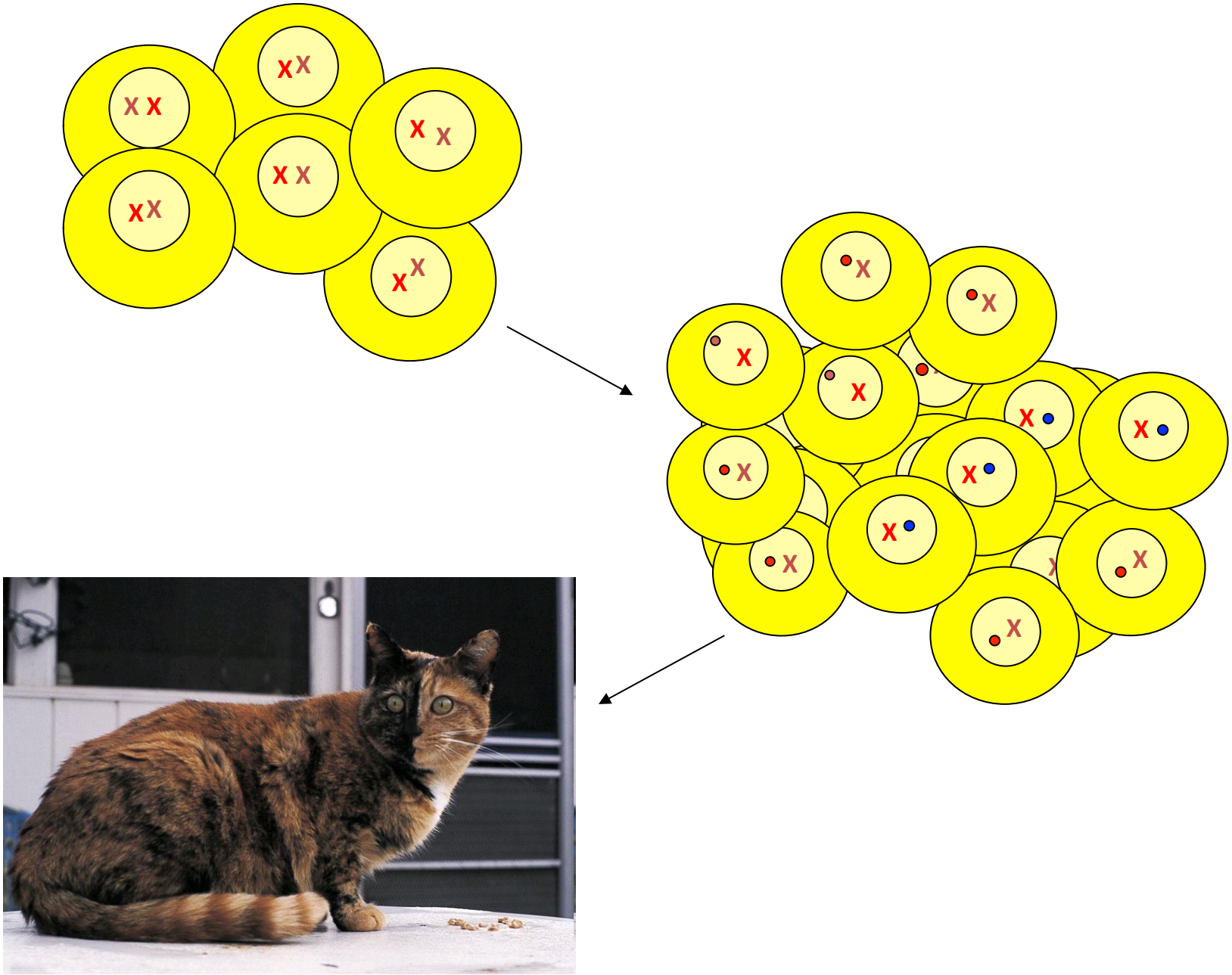
Barr body – One of the X chromosomes of women are inactivated as heterochromatin

During the development of a female embryo, one of the two X chromosomes are inactivated.

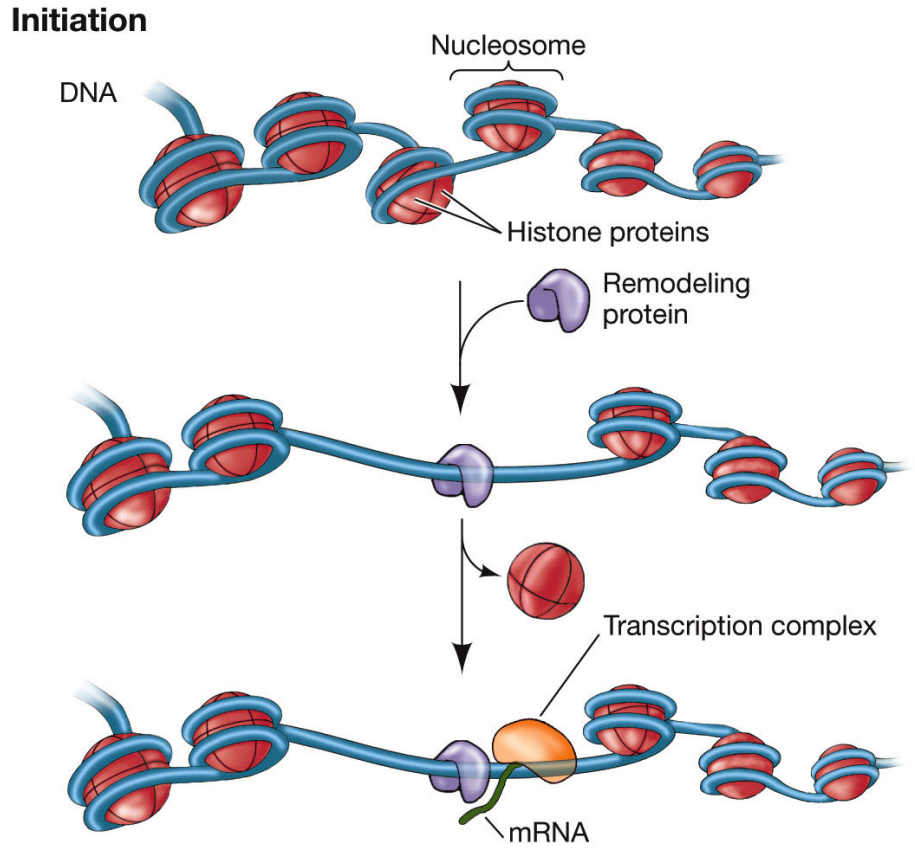


LIFE 8e, Figure 14.18

The female body is a mosaic, where some areas contain cells that have the one X chromosome inactivated, while cells in other areas have the other X chromosome inactivated



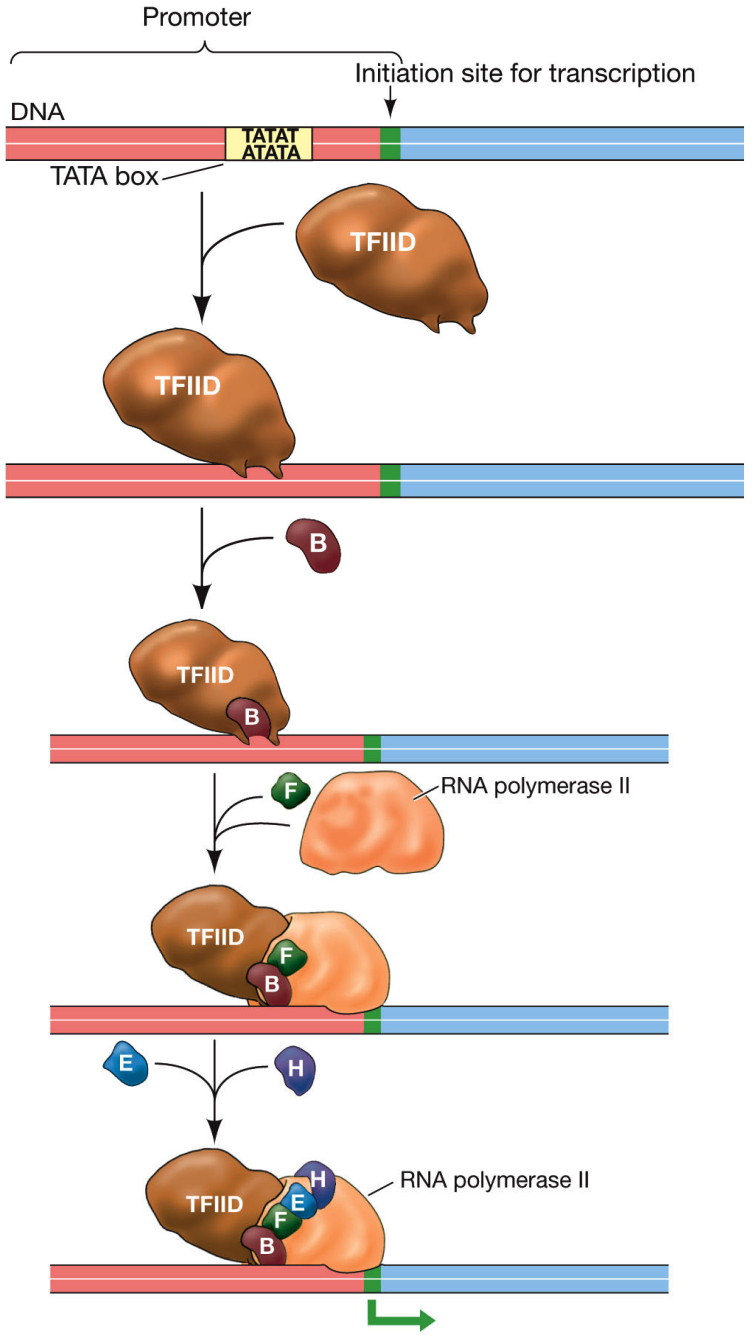
Remodelling chromatin structure before transcription



LIFE 8e, Figure 14.17 (Part 1)

Gene expression can be regulated here

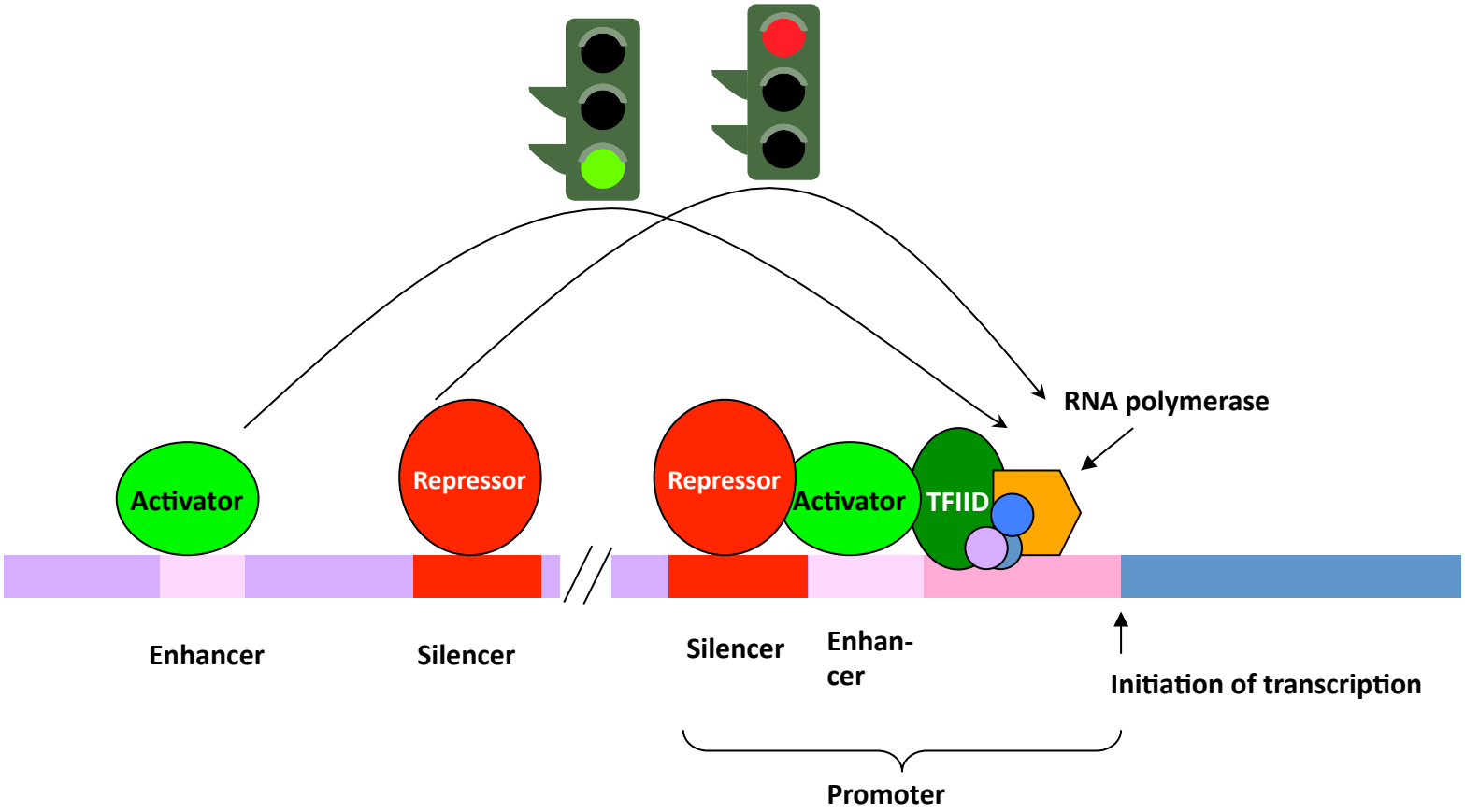
Initiating transcription



Gene expression can be regulated here

LIFE 8e, Figure 14.13 (Part 2)

More on initiation of transcription



The same transcription factor can bind/regulate several genes!

Gene expression can be regulated here

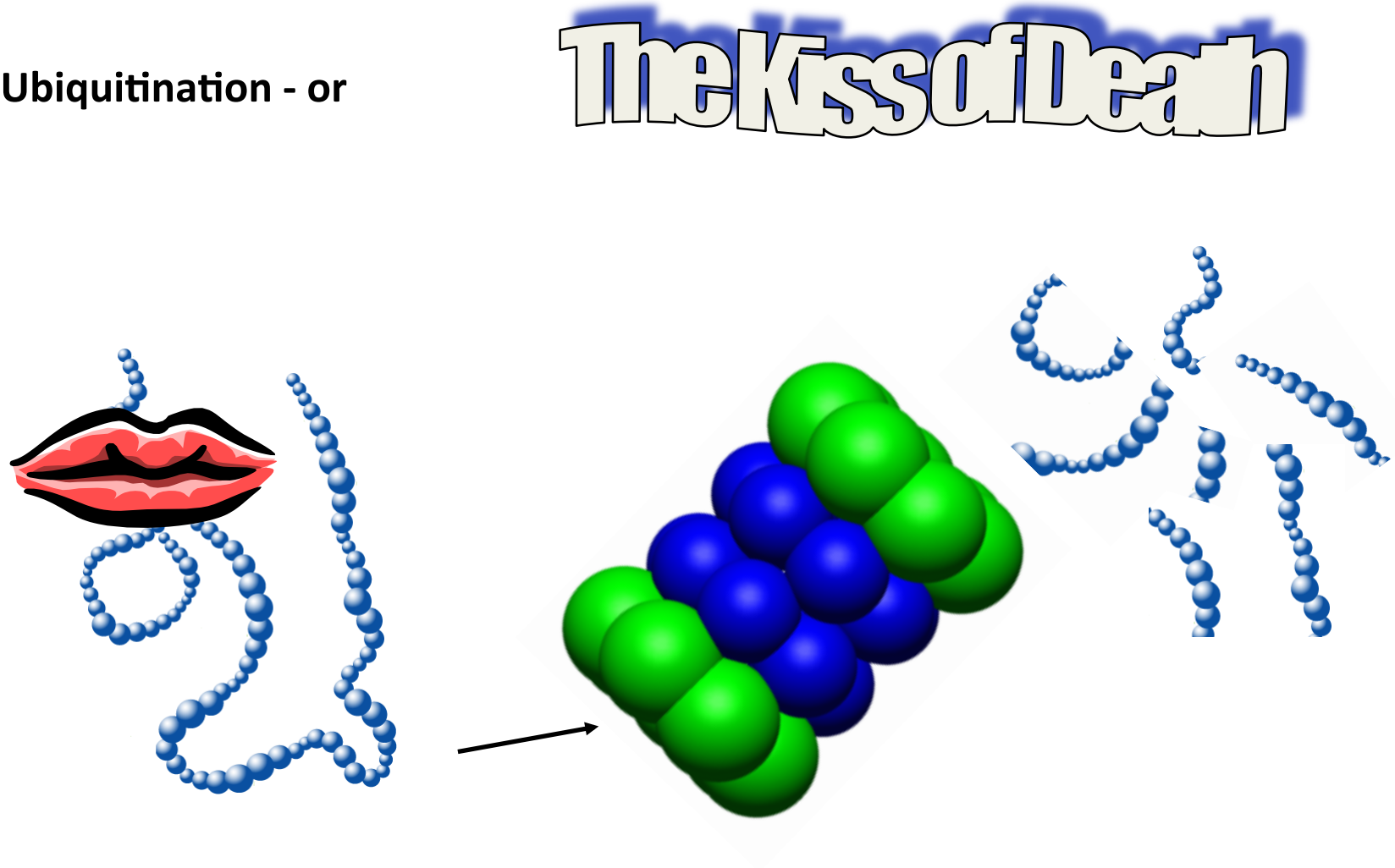
MicroRNA – regulation mRNA longevity

- Small RNA molecules, ≈ 22 to 23 nucleotides
- Bind via basepairing to the 3' end of the mRNA
- Binding inhibits translation and sometimes the mRNA is even degraded
- Computers predict that more than 1000 genes encode microRNA
- It is also predicted that microRNA molecules regulate the expression of more than 1/3 of all human genes

Gene expression can be regulated here

Post translationel regulation of expression

Ubiquitination - or



Gene expression can be regulated here

Learning objective

Describe how gene expression is regulated in eukaryotes emphasizing the many different levels this can be achieved on.

Typical exam question

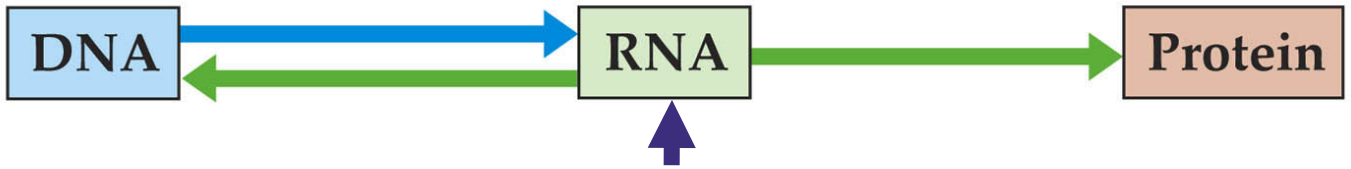
Q. A DNA sequence is several thousands of nucleotides distant from the promoter. When this sequence is bound by a protein, transcription rates increase greatly. This sequence is most likely a(n)

a. TATA box.
b. enhancer.
c. operon.
d. promoter.
e. consensus sequence.

DNA Microarray Technology

Eksempel på undtagelse

- Visse RNA virus, kaldet *retrovirus* (herunder HIV), oversætter deres RNA-genom "baglæns" til DNA efter at de har inficeret en celle
- Denne proces kaldes *revers transkription*, og foretages af enzymet *revers transkriptase*
- Retrovirusets DNA bliver integreret i værtscellens genom og kan ligge der i lang tid, før det bliver brugt til at danne nye virale RNA genomer ved almindelig transkription

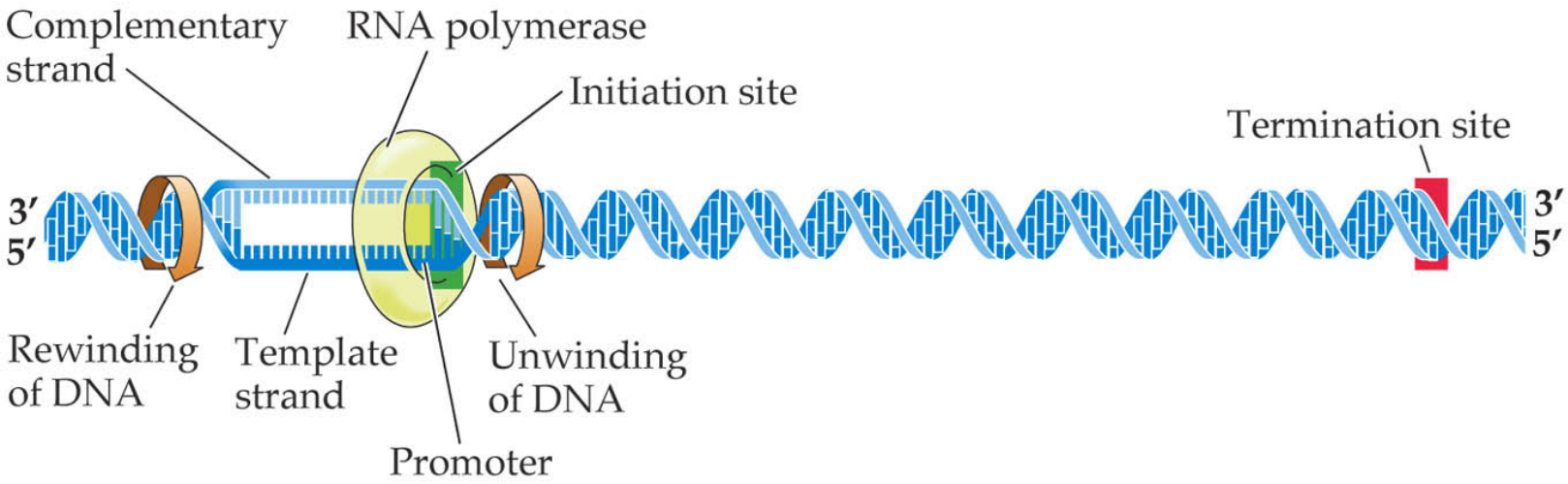


Arvematerialet

Forekommer også i forbindelse med *retrotransposons* (kap. 14)

Transkription: Initiering

- **Initiering** begynder ved en **promotor**, en særlig sekvens på DNA
- I eukaryoter er der (mindst) én promotor for hvert gen
- I prokaryoter kan flere gener aflæses fra samme promoter
- RNA polymerasen binder til promotor området
- Promotor sekvensen dirigerer hvilken streng RNA polymerasen skal bruge som template og dermed i hvilken retning den kører
- DNA dobbelt helixen skal delvist vikles ud for at fungere som template – dette gøres af RNA polymerasen



Transkription: Elongering

- RNA polymerase vikler DNA'et ud ca. 10 basepar ad gangen og læser template i 3' → 5' retning
- Efterhånden som RNA transkriptet dannes, frigøres det fra baseparring med DNA template strengen, og DNA går tilbage til dobbelthelix-konformationen
- Det nye RNA vokser i 5' → 3' retning; dvs. RNA transkriptet er antiparallelt til DNA template strengen
- RNA polymerasen kan ikke *korrekturlæse*: Transkriptionsfejl er relativt hyppige sammenlignet med DNA replikation